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Radiocarbon analysis of neutral sugars in high-molecular-weight dissolved organic carbon: Implications for organic carbon cycling

Daniel J. Repeta
Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Lihini I. Aluwihare
Geosciences Research Division, Scripps Institution of Oceanography, La Jolla, California 92037

Abstract

We used compound-specific natural-abundance radiocarbon analyses of neutral sugars to study carbon cycling of high-molecular-weight (HMW) dissolved organic carbon (DOC) at two sites in the North Pacific Ocean. Sugars released from HMW DOC by acid hydrolysis were purified by high-pressure liquid chromatography and analyzed for radiocarbon content via accelerator mass spectrometry. The seven most abundant sugars recovered from HMW DOC have similar radiocarbon values, supporting the hypothesis that these sugars are incorporated into a common family of polysaccharides. Neutral sugar $\Delta^{14}C$ values from surface waters collected in 1999 and 2001 are 89 ± 13‰ and 57 ± 6‰, respectively; these values are much more enriched in radiocarbon than those found in previous studies that used operationally defined carbohydrate fractions. Radiocarbon values for HMW DOC neutral sugars are the same as, or only slightly depleted relative to, dissolved inorganic carbon (DIC), which is consistent with rapid cycling and a short (<3-yr) residence time. In addition, the $\Delta^{14}C$ value of neutral sugars at 600 m is 20‰ enriched relative to DIC $\Delta^{14}C$, suggesting that a fraction of dissolved neutral sugars at this depth are introduced by dissolution from large, rapidly sinking particles.

Marine heterotrophic bacterial production is fueled by the metabolism of dissolved organic carbon (DOC) (Williams 2000). Numerous studies show that bacterial production is carbon limited, even though DOC concentrations are high throughout the water column (Kirchman 1990; Carlson and Ducklow 1995). The large reservoir of DOC that accumulates in seawater is therefore believed to be largely unavailable to meet bacterial carbon demand (Kirchman et al. 1993; Carlson and Ducklow, 1995). Radiocarbon measurements of deep-sea DOC support the view that a large fraction of DOC is recalcitrant (Williams et al. 1969; Williams and Druffel 1987; Druffel et al. 1992). Average radiocarbon values for DOC below 1,000 m at mid-gyre sites in the North Pacific and North Atlantic Oceans are −525 ± 20‰ and −390 ± 10‰ respectively, consistent with a residence time of 4,000–6,000 yr, or several ocean-mixing cycles (Druffel et al. 1992). However, at depths <1,000 m DOC concentrations begin to rise, and surface ocean DOC values can be up to 50 μmol L^{-1} higher than deep-sea concentrations (Hansell and Carlson 1998). The elevated concentrations and the shorter average residence time (~2,000 yr) of DOC in surface waters points to a large (30–50 GT C), recently synthesized DOC reservoir in the upper ocean. This reservoir is considered to be semireactive, turning over on timescales of upper-ocean mixing (months to decades). Because of the large size of the reservoir, semireactive DOC has the potential to satisfy a substantial fraction of annual bacterial carbon demand, but without a more exact estimate of its residence time, the role of semireactive DOC in bacterial metabolism cannot be fully evaluated.

Semireactive DOC is concentrated in the high-molecular-weight fraction of DOC (HMW DOC). Between 20% and 35% of total DOC is colloidal or HMW DOC and can be isolated by ultrafiltration for isotopic measurements. HMW DOC has higher (more modern) $\Delta^{14}C$ values than total DOC except in some deep-water samples influenced by the benthic boundary layer (Guo et al. 1996; Guo and Santschi 1997). Nuclear magnetic resonance (NMR) spectra of HMW DOC show that most of the carbon, at least 50–70%, is carbohydrate. The remaining carbon is a mixture of proteins (3–5% HMW DOC), lipids (1% HMW DOC), and uncharacterized humic substances. Both the absolute and relative amounts of carbohydrate in HMW DOC decrease with depth (Aluwihare et al. 2002), in parallel with the loss of semireactive DOC, indicating that carbohydrates are a large part of the semireactive fraction of HMW DOC. Therefore, to obtain an estimate of semireactive DOC residence time without interference from the recalcitrant DOC fraction, several recent studies have reported radiocarbon measurements of carbohydrate fractions isolated from HMW dissolved organic matter (HMW DOM).

Santschi et al. (1998) used ethanol precipitation to concentrate the carbohydrate fraction from surface HMW DOM sampled in the Middle Atlantic Bight and found that this fraction was 138‰ enriched in ^{14}C relative to HMW DOC ($\Delta^{14}C$ of 26‰ vs. −112‰). No enrichment was found for ethanol precipitates of deep-sea HMW DOC samples, which are known to have much lower amounts of carbohydrate.

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More recently, Loh et al. (2004) used ion exchange chromatography and acid precipitation to isolate a ''carbohydrate-like'' fraction from HMW DOC. The isotopic value of carbohydrate-like fractions isolated from surface water HMW DOC ranged from 7‰ to 13‰ and were enriched in radiocarbon by 18–97‰ over HMW DOC $\Delta^{14}C$ values, in agreement with the results of Santschi et al. (1998). Expected surface water DIC $\Delta^{14}C$ values at the study sites are significantly greater than the $\Delta^{14}C$ values reported for HMW DOC carbohydrate-like fractions, suggesting slow cycling and a long residence time for HMW DOC carbohydrates (>several decades). However, as carbohydrates were not directly identified or quantified in fractions that were analyzed for radiocarbon, the relative depletion in $\Delta^{14}C$ observed in both studies could be due to the inclusion of radiocarbon-depleted, noncarbohydrate impurities in the carbohydrate-like fraction.

One potential solution for addressing the uncertainties inherent in radiocarbon measurements made on operationally defined HMW DOM fractions is to make compound-specific radiocarbon measurements on neutral sugars that comprise a major fraction of dissolved carbohydrate. NMR and molecular-level (gas chromatography–mass spectrometry, high-pressure liquid chromatography [HPLC]) analyses reveal that 10–20% of HMW DOC is a suite of seven neutral sugars, and that the distribution of these sugars is conservative in samples from different geographic locations and depths in the ocean (McCarthy et al. 1996, Aluwihare et al., 1997). HMW DOC with similar characteristics is produced by marine phytoplankton in culture, and Aluwihare et al. (1997) concluded that phytoplankton synthesize a specific family of closely related acylated polysaccharides (APS) that accumulate as semireactive DOC in seawater. APS is distinguished by a novel suite of seven neutral sugars and a high relative abundance of N-acetylated sugars (Aluwihare et al. 2005). In an earlier study of dissolved carbohydrates in the NW Atlantic Ocean, we measured the $\Delta^{14}C$ value of three neutral sugars (rhamnose, fucose, xylose) isolated from HMW DOC by acid hydrolysis and purified by HPLC (Aluwihare et al. 2002). Neutral sugar values ranged from 49‰ to 92‰, with an average value of 71 ± 30‰, comparable to the value of dissolved inorganic carbon (DIC) $\Delta^{14}C$ of 59‰. Unlike other reports using operationally defined carbohydrate fractions, we found purified carbohydrates to have radiocarbon values that suggest very rapid, annual cycling of semireactive DOC. If additional measurements show that semireactive DOC is cycling on annual rather than decadal timescales, then this reservoir of carbon supports a significant fraction of annual bacterial carbon demand in the ocean.

Methods

Ultrafiltration equipment, glassware, and Teflon™ products were purchased expressly for this study and had no history of exposure to tracer-level radiocarbon contamination. Glassware was combusted at 450°C overnight before use. Teflon products were cleaned by soaking in concentrated nitric acid for at least 24 h. All manipulations were carried out in the Fye Organic Geochemical Laboratory, a radiocarbon-free building at the Woods Hole Oceanographic Institution. Water for cleaning and diafiltration was ultrapure (Milli-Q) grade. Hydrochloric acid, NH_4OH, and NaOH were reagent-grade ACS.

Large-volume seawater samples were drawn from the 15-m and 600-m intake pipe at the Natural Energy Laboratory in Kona, Hawaii in February 2002 (Hawaii 15 m, 600 m). The samples were filtered to remove bacteria and small particles using a cleaned (10% HCl) Suporflow dual-stage (0.8 μm and 0.2 μm) Gelman polyether sulfone cartridge filter (Chisolm) fitted to an Advanta stainless-steel housing. HMW DOM samples were collected using a cross-flow ultrafiltration system consisting of a stainless-steel centripetal pump and membrane housings and a fluorinated high-density polyethylene reservoir. The system was plumbed with Teflon tubing and fitted with polyvinyl difluoride valves. The ultrafiltration membrane (Separation Engineering) nominally retains organic matter with a relative molecular weight of >1 kDa (>99% rejection of vitamin B_{12}). Membranes were cleaned using isopropanol, detergent (0.01% micro), HCl (0.01 mol L^{-1}), and NaOH (0.01 mol L^{-1}); stored in sodium azide (0.55 mmol L^{-1}); and rinsed with water immediately before use. Between 30,000 and 60,000 liters of seawater were concentrated to approximately 20 liters, frozen in fluorinated high-density polyethylene containers, and returned to Woods Hole for further processing. Samples were desalted by diafiltration with water, reduced to 2 liters, and lyophilized to a fluffy white powder.

A sample of surface seawater (3–5 m) was also collected from the North Pacific Subtropical Gyre (NPSG) (31°00'N, 159°00'W) in June 1999. The sample was taken contemporaneously with the 20-m Pacific Ocean HMW DOC sample reported in Loh et al. (2004). Seawater was collected using an air-driven diaphragm pump fitted with Teflon tubing deployed over the side of the ship and filtered (0.2 μmol L^{-1} Criticap polycarbonate filter cartridges) before being ultrafiltered using a spiral wound 1 kDa nominal molecular weight cutoff filter (Amicon Corp.) mounted on an Amicon DC-10 pump (Aluwihare et al. 2002). Sample concentrates (2–4 liters) were stored in 2-liter Teflon bottles, frozen at −20°C, and returned to Woods Hole for processing as described above.

Monosaccharides were isolated from 0.5 g HMW DOM after acid hydrolysis with 4 mol L^{-1} HCl (300 mL) at 108°C for 4 h. Following the hydrolysis, samples were cooled and frozen before the acid was removed by freeze drying. The residue was dissolved in approximately 4 mL of water and desalted using Biorex 5 anion exchange resin (4 g, 100–200 mesh, OH^- form, BioRad). The column was washed with 30 mL of ultrahigh-purity water to elute a carbohydrate fraction. A second fraction containing amino acids and molecularly uncharacterized carbon (MUC) was eluted next with 22 mL of 2 mol L^{-1} NH_4OH. The carbohydrate fraction was freeze-dried, dissolved in 1–2 mL of water, and further purified by HPLC with refractive index detection using two cation exchange columns (0.8 × 30 cm, sulfonated styrene–divinyl benzene in Ag^{2+} form [Supelcogel™ Ag]) connected in series. Neutral sugars elute in approximately 30–45 min, using a flow rate of 0.5 mL min^{-1} of high-purity water at 80°C (Fig. 1A). Monosaccharides were collected in three

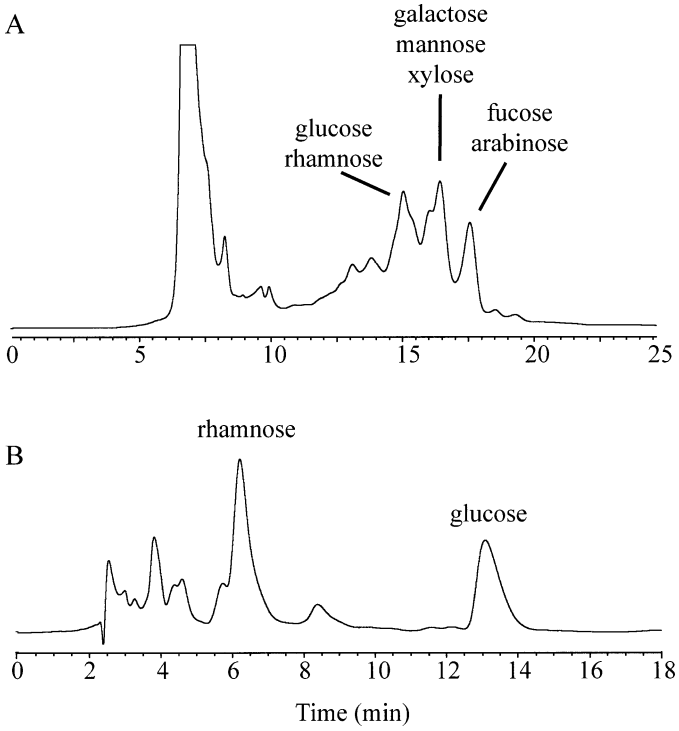

Fig. 1. (A) Separation of neutral sugars from HMW DOC by ion exchange HPLC after acid hydrolysis. Neutral sugars elute in three fractions (F1–F3; F1 glucose, rhamnose; F2 glactose, mannose, xylose; and F3 fucose, arabinose). The three fractions are collected separately, dried, and further purified by reverse-phase HPLC (B) using a polymeric amino column before radiocarbon analyses. The chromatogram shows the separation of fraction 1 (glucose, rhamnose) from the Hawaii surface-water sample.

fractions: F1 (glucose, rhamnose), F2 (galactose, mannose, xylose), and F3 (fucose, arabinose). Each fraction, freeze-dried, was dissolved in 20–40 μL of water, diluted to 100–200 μL with acetonitrile, and further purified by reverse-phase HPLC using two amino columns (Hamilton PXP-700) connected in series and eluted at 1 mL min^{-1} with 80 : 20 acetonitrile : water (Fig. 1B). Proton NMR (^{1}H NMR) spectra were collected on a Bruker 400 MHz spectrometer with water suppression (zgpr). All spectra were collected using D_2O as a solvent and chemical shifts referenced to water at 4.8 ppm.

Purified sugars were collected, evaporated to dryness, dissolved in 1 mL of water, and transferred to a combusted (850°C, 5 h) 8'' Vycor quartz tube (9 mm o.d., 7 mm i.d.) containing 300–500 mg of copper oxide (Elemental Microanalyses). The samples were lyophilized overnight and sealed under vacuum for combustion. Sealed tubes were combusted at 850°C for 5 h, and the carbon dioxide generated during combustion was quantified, transferred to a 6-mm pyrex tube, and submitted to the National Ocean Sciences Accelerator Mass Spectrometry facility in Woods Hole, Massachusetts for natural abundance radiocarbon analyses.

Results and discussion

Compound-specific radiocarbon analyses and DOC cycling in surface seawater—To measure the residence time of dissolved carbohydrates, we used compound-specific radiocarbon analyses of neutral sugars extracted from HMW DOC. Table 1 reports values for DIC Δ^{14}C, HMW DOC Δ^{14}C, and neutral sugar Δ^{14}C in samples collected at the study site near Kona, Hawaii. Our Hawaii surface seawater sample has a DIC Δ^{14}C value of 72 $\pm$ 7‰ and shows the incorporation of bomb radiocarbon into surface-water DIC. Radiocarbon is supplied to the ocean by CO_2 exchange with the atmosphere, which is enriched in ^{14}C from natural and

Table 1. Isotopic composition of bulk carbon fractions and neutral sugars from Hawaii. The δ^{13}C values are given relative to Pee Dee Belemnite (PDB). Δ^{14}C values were determined by accelerator mass spectrometry and are corrected for isotopic fractionation. We assumed a value of −20‰ for sugars with undetermined δ^{13}C values. The isotopic enrichment in the δ^{13}C value of xylose relative to glucose is similar to that reported by van Dongen et al., 2002 for aquatic and terrestrial plants. Columns containing two values show the results of duplicate analyses of samples split immediately before combustion.

	Hawaii (15 m)		Hawaii (600 m)	
Sample	δ^{13}C (‰)	Δ^{14}C (‰)	δ^{13}C (‰)	Δ^{14}C (‰)
DIC*	1.37	72±7 (n=4)	−0.21	−155±7 (n=4)
HMW DOC	−21.9	10	−20.9	−262, −255
F1		49		
F2		36		
Glucose	−19.9	47, 58		−133
Galactose		67		−108
Mannose		65		−121
Xylose	−15.6	52, 58		−129
Arabinose		63		
Fucose	−18.8	49, 52		
Rhamnose		40, 57		

* DIC, dissolved inorganic carbon; HMW DOC, high-molecular-weight dissolved organic carbon.

anthropogenic (above ground testing of nuclear weapons in the 1950s and 1960s) production. Bomb-produced radiocarbon raised marine DIC $\Delta^{14}C$ values from between −50‰ and −80 ‰ before 1945 to above 150‰ at some North Pacific Ocean sites in the mid 1970s (Druffel 1987; Mahadevan 2001). As a result of vertical mixing with radiocarbon-depleted subsurface water, surface seawater DIC $\Delta^{14}C$ values are now decreasing and this is reflected in the DIC $\Delta^{14}C$ value of our surface-water sample.

Hawaii surface-water HMW DOC $\Delta^{14}C$ is 10‰, significantly depleted in radiocarbon relative to DIC. Purification of neutral sugars by HPLC yields mixed sugar fractions with $\Delta^{14}C$ values of 36‰ and 49‰, intermediate between DIC $\Delta^{14}C$ and HMW DOC $\Delta^{14}C$ (F1, F2; Table 1). Both HMW DOC and neutral sugar isolates show the incorporation of bomb radiocarbon, indicating that each fraction contains compounds synthesized over the past 50 yr. Further purification by reverse-phase HPLC yields seven neutral sugars with radiocarbon values of 47–67‰. In an earlier paper, we suggested that the seven neutral sugars isolated from HMW DOM are incorporated into a common family of structurally related algal biopolymers that are selectively preserved as semireactive HMW DOM (Aluwihare et al. 1997). Proton and ^{13}C NMR spectra of HMW DOM isolated from diverse locations in the North and South Pacific Ocean, and the North Atlantic Ocean, including coastal and open ocean sites, show remarkable similarity in the relative amounts of carbon and hydrogen in major functional groups (COOH, HCOH, $COCH_3$, CH_x). Likewise, hydrolysis of HMW DOM samples yields the same suite of seven neutral sugars in relatively fixed proportions (McCarthy et al. 1996; Aluwihare et al. 1997; Eglinton and Repeta 2004). HMW DOM with similar NMR spectral characteristics and neutral sugar distributions has been recovered after bacterial degradation of DOM produced in laboratory algal cultures. The conservation of functional group and neutral sugar distributions in HMW DOM across many different oceanographic regimes in samples collected over a 10-yr time span, along with the production of HMW DOM with similar characteristics in pure cultures, was taken as evidence for selective preservation of a novel biopolymer in HMW DOM.

The similarity in radiocarbon values for different neutral sugars is consistent with our earlier suggestion that these sugars are incorporated into a common family of biopolymers (APS; Aluwihare et al. 1997). If neutral sugars were part of a common biopolymer, we would expect them to have the same carbon sources and diagenetic history, and therefore the same isotopic values within the range of normal cellular isotopic heterogeneity for carbohydrates (3–5‰ for ^{13}C), with an isotopic enrichment for C5 sugars (van Dongen et al. 2002). The radiocarbon data alone do not exclude the possibility that neutral sugars are derived from a mixture of different polysaccharides; however, along with previously published data on functional group (NMR) and monosaccharide distributions in HMW DOM, the similarity in radiocarbon values of neutral sugars does support the hypothesis that neutral sugars are bound together in a common family of biopolymers.

Assuming the radiocarbon determinations of individual sugars in HMW DOC serve as replicates, then the average $\Delta^{14}C$ for neutral sugars in HMW DOC is 57 ± 6‰ (1 SD, $n = 11$). This value is only slightly depleted in ^{14}C relative to DIC, indicating very recent synthesis of these sugars in surface waters. We believe that the small depletion in the neutral sugar $\Delta^{14}C$ value most likely results from incomplete HPLC purification of individual sugars. Our neutral sugar data are not corrected for blanks. Neutral sugar isolates typically contained 30–50 μmol of carbon per sugar. Procedural blanks were found to have 0.5–1 μmol of carbon for each sugar collected, representing 1–2% of the total carbon analyzed. Procedural blanks were too small to measure radiocarbon directly, so we made radiocarbon measurements on composite blanks by pooling the blanks of all individual sugars. Composite blanks were found to have radiocarbon values of approximately −700‰ to −800‰. If neutral sugars have the same radiocarbon value as DIC (72‰), we expect the value of 98–99% purified neutral sugars to have a radiocarbon value that is 7‰ to 16‰ depleted from DIC or about 56–65‰.

Table 2. Isotopic composition of bulk carbon fractions and neutral sugars at 3 m in the North Pacific Subtropical Gyre. The data for DIC $\Delta^{14}C$ were kindly provided by Dr. Ellen Druffel.

Sample	$\Delta^{14}C$ (‰)
DIC	89±7
HMW DOC	46
Glucose	79
Galactose	103
Mannose	99
Xylose	94
Arabinose	ND*
Fucose	69
Rhamnose	57

* ND, not determined due to insufficient sample; DIC, dissolved inorganic carbon; HMW DOC, high-molecular-weight dissolved organic carbon.

We measure the same trends in the radiocarbon distribution for DIC, HMW DOC, and neutral sugars collected in the NPSG (Table 2). Surface seawater DIC $\Delta^{14}C$ is 89 ± 7‰, (Druffel pers. comm.) and is enriched relative to HMW DOC $\Delta^{14}C$ (46‰). Neutral sugars purified from HMW DOM have radiocarbon values between 67‰ and 103‰, and an average value of 89 ± 13‰, equal to DIC $\Delta^{14}C$. As with the Hawaii sample, we find very similar radiocarbon values for each neutral sugar, consistent with the hypothesis that these sugars are part of a common bioploymer.

Compound-specific radiocarbon analyses provide a different view of carbon cycling from previous studies that measured radiocarbon in operationally defined fractions of HMW DOC. All previous measurements made on operationally defined carbohydrate-like fractions of HMW DOC show this fraction to be significantly depleted relative to DIC $\Delta^{14}C$, consistent with slow cycling and turnover times of several decades (Santschi et al. 1998; Loh et al. 2004). Using the compound-specific approach we find carbohydrates to have radiocarbon values equal to or at most only slightly depleted from DIC, consistent with rapid cycling on annual rather than decadal timescales (see next section).

Williams and Druffel (1987) have modeled upper-ocean DOC $\Delta^{14}C$ values as simple two-component mixtures of old

and new carbon. Their model assumes that old DOC has a concentration and radiocarbon value equal to deep-sea DOC, whereas the new component includes DOC in excess of deep-sea concentrations and has a radiocarbon value equal to DIC $\Delta^{14}C$. Using this approach, Williams and Druffel (1987) showed that the measured isotopic value for DOC in North Pacific Ocean surface water (87 μmol L^{-1}; −146‰) could be modeled as a mixture of recently synthesized DOC (49 μmol L^{-1} C or 56% total DOC; $\Delta^{14}C$ = 150‰), and old DOC (38 μmol L^{-1} C or 44% total DOC; $\Delta^{14}C$ = −525‰) upwelled from the deep ocean (model result = −147‰). A similar calculation for the North Atlantic Ocean (Sargasso Sea) using the data of Druffel et al. (1992) yields a value for upper-ocean DOC $\Delta^{14}C$ of −120‰, indistinguishable from the measured value of −127‰. These radiocarbon data support the conclusion that upper-ocean DOC is a mixture of old, recalcitrant DOC and new, semireactive DOC. However, on the basis of these measurements, the residence time of the semireactive fraction cannot be determined to better than a few decades.

The two-component model used by Williams and Druffel (1987) predicts upper-ocean DOC $\Delta^{14}C$ values quite well, but the major assumption of the model, that there is an old component of DOC with a radiocarbon value equal to deep-sea DOC $\Delta^{14}C$, and a new component of DOC with a radiocarbon value equal to DIC $\Delta^{14}C$, has never been verified by direct measurements. Our data for HMW DOC neutral sugars substantiates the assumption that a fraction of upper-ocean DOC has a radiocarbon value equal to DIC. To measure the radiocarbon value of the old component, we analyzed an MUC fraction isolated by ion exchange chromatography from HMW DOC. Ten percent of HMW DOC from the NPSG site is retained by the Biorex anion ion exchange resin, but eluted by 2 mol L^{-1} NH_4OH (22 mL). This fraction has spectral characteristics nearly identical to MUC found in deep-sea HMW DOC (Fig. 2), and has a $\Delta^{14}C$ of −416‰. Our $\Delta^{14}C$ value for MUC in surface water is within the range of values for total HMW DOC isolated from 900 to 5,200 m at this site (−380‰ to −440‰, Repeta unpubl. data; Loh et al. 2004), and therefore verifies the existence of an old fraction of DOC in surface water with radiocarbon values similar to deep-sea DOC.

The agreement between observed and modeled values for DOC $\Delta^{14}C$ reported by Williams and Druffel (1987) and Druffel et al. (1992) along with our measurements of the radiocarbon values for neutral sugars and MUC suggest that most upper-ocean HMW DOC is a mixture of new ($\Delta^{14}C$ = DIC) and old ($\Delta^{14}C$ = deep-sea HMW DOC) carbon. Our data imply that previous reports of DOC fractions with $\Delta^{14}C$ values intermediate between new (surface DIC) and old (deep-sea DOC) likely reflect different proportions of old and new DOC within a particular fraction isolated for analyses. Although analyses of a much broader suite of organic HMW DOC components may ultimately yield some constituents with a range of radiocarbon values, to date we have not found evidence in support of a continuum of radiocarbon values for different components of DOC. Our data also show that both new (labile) and old (refractory) components exist within HMW DOM and as such, do not support a molecular size-dependent continuum of organic carbon diagenesis

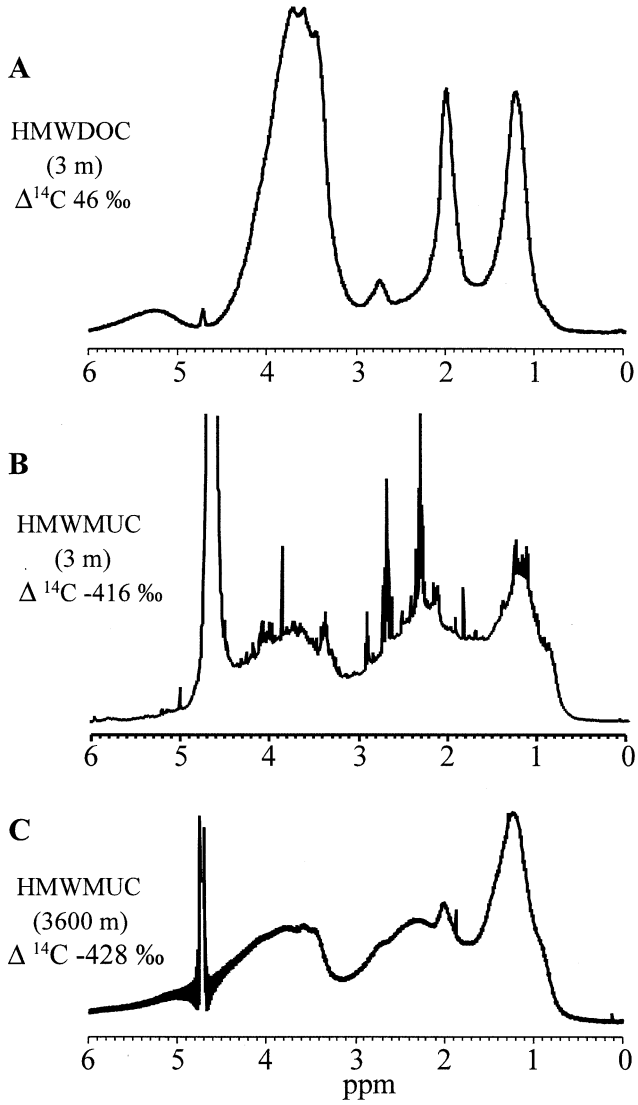

Fig. 2. (A) ^{1}H NMR spectrum of HMW DOC isolated from the NPSG (3 m) with a $\Delta^{14}C$ value of 46‰. Major functional groups include OC**H**O (carbohydrate, 5–6 ppm), **H**COH (carbohydrate, 3–4.5 ppm), OCC$\mathbf{H}_3$ (acetate, 2 ppm), and alkyl carbon (C$\mathbf{H}_x$, 1.3 ppm). The ratio of these functional groups is relatively fixed in HMW DOM from surface seawater. (B) The MUC fraction of HMW DOC (HMW-MUC) isolated by ion exchange chromatography. Approximately 5–10% of the carbon in the MUC fraction is from amino acids released during the hydrolysis of dissolved proteins and which appear as sharp well-defined peaks in the ^{1}H NMR spectrum (Quan and Repeta unpubl. data). The MUC component has spectral characteristics similar to deep-sea HMW DOC collected at 3,600 m (C), as well as a similar radiocarbon value (−416‰ for MUC isolated from surface seawater compared to −428‰ for HMW DOC at 3,600 m). The relative amounts of carbohydrate (3–4.5 ppm) **α**-functionalized alkyl (1.5–3 ppm) and alkyl carbon protons (1–1.5 ppm) are similar in the two spectra. NMR spectra were collected at 400 MHz in D_2O using solvent suppression (zgpr) and referenced to water at 4.8 ppm.

(Amon and Benner 1996; Loh et al. 2004). We suggest that the observed depletion in total DOC $\Delta^{14}C$ relative to HMW DOC $\Delta^{14}C$ results from the higher proportion of low-molecular-weight (LMW) humic substances in total DOC, as com-

pared with the newly synthesized carbohydrate-rich APS fraction that is concentrated in HMW DOC. For example, marine humic substances isolated by adsorption onto polystyrene XAD resins have an average relative molecular weight <1 kDa, and have been shown to be highly depleted in radiocarbon (Stuermer and Harvey 1974; Druffel et al. 1992). In contrast, APS is abundant in HMW DOC fractions from >1 kDa to >10 kDa (Eglinton and Repeta 2004) and neutral sugars data provided here show APS to be modern with radiocarbon values similar to DIC. Therefore, differences in chemical composition (rather than molecular size) likely control differences in oceanic residence time, but chemically distinct components are unevenly distributed within HMW and LMW DOC.

Bacterial production and the cycling of APS—Our radiocarbon measurements can be used to estimate the turnover of dissolved neutral sugars in seawater, and evaluate their contribution to annual bacterial production. Marine bacterial production is thought to be supported by the respiration of highly reactive DOC that is consumed on timescales of hours to days, and maintained at low steady-state concentrations (Carlson 2002; Cherrier and Bauer 2004). The global inventory of a semireactive fraction of DOC that accumulates at depths <800–1,000 m is very large (30–50GT C), but is not thought to fuel a significant fraction of bacterial carbon demand (Kirchman et al. 1993; Carlson and Ducklow 1995). Loh et al. 2004 measured radiocarbon values for protein-like and carbohydrate-like fractions of HMW DOC in surface water and found both fractions to be considerably aged ($\Delta^{14}C$ values between 2‰ [protein-like HMW DOC] and 13‰ [carbohydrate-like HMW DOC] and −2‰ and 7‰ at their Pacific and Atlantic Ocean sites, respectively). Such values are consistent with a residence time of several decades (>50 yr) and slow cycling of semireactive HMW DOC, which implies that this fraction satisfies only a small fraction (<1 GT C yr^{-1}) of annual bacterial carbon demand. The slow cycling of HMW DOM is further supported by the slow temporal evolution of semireactive DOC profiles in the upper ocean.

The value of DIC $\Delta^{14}C$ for surface waters in the NPSG is not currently at steady state, but is changing due to a decreasing atmospheric $\Delta^{14}C$ value, vertical mixing (convection and diffusion), and horizontal advection (Mahadevan 2001). The history of radiocarbon changes in the NPSG are known from high-resolution $\Delta^{14}C$ measurements of corals, and from data collected as part of the global ocean carbon survey programs. Coral-derived records show that for the century before 1952, NPSG surface water was in steady state with respect to long-term mixing with atmospheric $\Delta^{14}C$, as shown by the 7‰ decrease in $\Delta^{14}C$ between 1893 and 1952 induced by the Seuss effect (dilution of atmospheric^{14}C with dead carbon from fossil fuel combustion; Druffel et al. 2001). High-resolution coral records additionally show seasonal changes in $\Delta^{14}C$ of approximately 7‰ near Hawaii due to wind-driven changes in upper-ocean mixing. DIC $\Delta^{14}C$ data have been modeled using the three-dimensional Massachusetts Institute of Technology (MIT) general circulation model to distinguish processes that affect the time series evolution of $\Delta^{14}C$ in the Pacific Ocean. These simulations suggest that near our NPSG study site, $\Delta^{14}C$ was decreasing at about 4‰ per year in the late 1990s and that the major process affecting surface-water DIC $\Delta^{14}C$ (up to 1995) is convection within the mixed layer (Mahadevan 2001). This agrees well with direct DIC $\Delta^{14}C$ measurements taken at the NPSG site in 1987 and 1999 that show an average annual decrease in $\Delta^{14}C$ of 4‰ between 1987 (DIC $\Delta^{14}C$ = 131‰; Druffel et al. 1992) and 1999 (DIC $\Delta^{14}C$ = 89‰; Druffel pers. comm.).

To simulate the effect of a changing value of DIC $\Delta^{14}C$ on the semireactive fraction of HMW DOC as suggested by the Williams and Druffel model, we constructed a simple box model of semireactive $\Delta^{14}C$ in the upper ocean (Fig. 3). Our model assumes a prebomb (1959) value of DIC $\Delta^{14}C$ and semireactive HMW DOC $\Delta^{14}C$ of −80‰ in the NPSG (Mahadevan 2001). After this date, as DIC $\Delta^{14}C$ values begin to rise, the isotopic enrichment is transferred into the semireactive HMW DOC at a rate determined by the residence time of the semireactive HMW DOC. Short residence times for semireactive HMW DOC result in rapid transfer of postbomb carbon into HMW DOC, whereas longer residence times result in slow transfer of bomb radiocarbon into HMW DOC (Fig. 3A). By assuming different residence times for the semireactive HMW DOC fraction, we can compare the value of semireactive HMW DOC $\Delta^{14}C$ with DIC $\Delta^{14}C$. The model assumes that the concentration of HMW DOC is at steady state, that the same fractional amount of HMW DOC is replaced annually, and that there is no isotopic discrimination in the removal of semireactive HMW DOC. The $\Delta^{14}C$ value of semireactive HMW DOC (HMW DOC_{SR}) for any year (t) is therefore expressed as: HMW DOC_{SR} $\Delta^{14}C(t)$ = DIC $\Delta^{14}C(t)(k)$ + HMW $DOC_{SR}(t - 1)(1 - k)$, where t is the year and k is the fraction of HMW DOC_{SR} replaced annually (e.g., if the residence time is 10 yr, k = 1/10 or 0.1). If the residence time of HMW DOC_{SR} is short (1–3 yr), there is little difference between the isotopic value of DIC $\Delta^{14}C$ and HMW DOC_{SR} $\Delta^{14}C$ ($\Delta\Delta^{14}C$; Fig. 3B). As residence times for HMW DOC_{SR} become longer, the HMW DOC_{SR} fraction becomes progressively depleted in $\Delta^{14}C$ relative to DIC $\Delta^{14}C$ and $\Delta\Delta^{14}C$ increases (Fig. 3A).

DIC and neutral sugars at the NPSG site have the same radiocarbon value within the uncertainty of our measurements (±13‰), consistent with a residence time of <1–3 yr or between 20 and 25 yr (Fig. 3). Because DIC had a $\Delta^{14}C$ value of 89‰ both before and after the ocean radiocarbon maximum in the late 1970s, our measurements at one time point do not allow us to determine a single residence time for neutral sugars. However, additional measurements of neutral sugars $\Delta^{14}C$ values on samples collected over the next several years would allow for a distinction between the <3 yr and 20–25 yr residence times. If neutral sugars had residence times between 4 and 20 yr, they would have $\Delta^{14}C$ values measurably greater than present-day DIC $\Delta^{14}C$ because during this time period DIC $\Delta^{14}C$ values in the surface ocean were higher because of the penetration of the atmospheric bomb ^{14}C signal. If neutral sugar residence times were >20–25 yr, sugar $\Delta^{14}C$ values in 1999 would have been significantly lower than DIC $\Delta^{14}C$. Our results show that neutral sugars have residence times that are at least a factor of 2–3, but perhaps over an order of magnitude shorter than

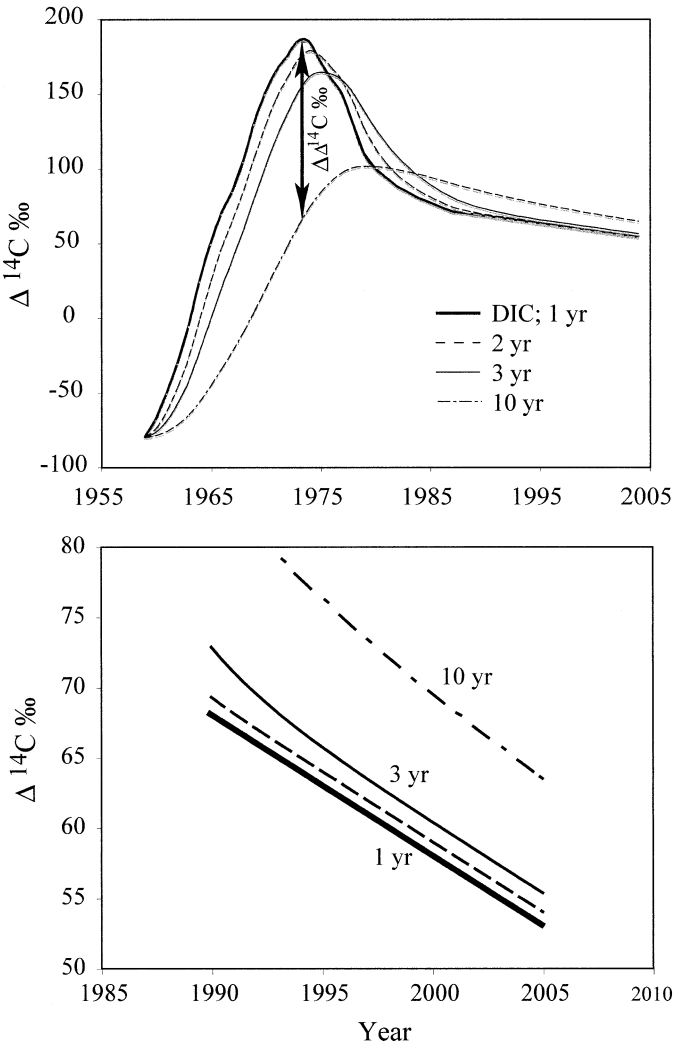

Fig. 3. (A) Results from a box model of semireactive HMW DOC in surface seawater of the NPSG between 1959 and 2002. The model assumes a prebomb DIC $\Delta^{14}C$ value of −80‰. DIC $\Delta^{14}C$ values then rise to their maximum in 1973–1974, and decrease thereafter. As bomb radiocarbon is transferred into semireactive HMW DOC, HMW DOC $\Delta^{14}C$ values also begin to rise at a rate dependent on the assumed residence time of semireactive HMW DOC (1, 2, 3, and 10 yr in the figure). The difference in DIC $\Delta^{14}C$ and semireactive HMW DOC $\Delta^{14}C$ ($\Delta\Delta^{14}C$) is determined by the residence time of the semireactive HMW DOC fraction. (B) Expansion of the model output for 1990–2005. Radiocarbon values for DIC were taken from Pearson (2000).

suggested by previous radiocarbon measurements made on operationally defined carbohydrate fractions.

Our radiocarbon-derived residence time for semireactive HMW DOC can be compared with estimates of residence time derived from spatial and temporal changes in DOC inventory in the NPSG. Export of DOC either below the euphotic zone or away from high-productivity regions decouples DOC production from heterotrophic consumption, thereby providing a complementary means for measuring semireactive DOC residence time. Abell et al. (2000) followed the degradation of total organic carbon (TOC) along isopycnals that outcrop within the NPSG between 15 and 30°N and 150–160°W. They found that TOC at depths <200 m had a residence time of 5 yr, whereas TOC at depths of 200–300 m had a residence time of 13 yr. By comparing TOC and total organic nitrogen degradation, Abell et al. (2000) further showed that the remineralized organic matter was poor in nitrogen, with an a C : N of 30 ± 10, suggesting a carbohydrate-rich substrate. These estimates of DOC respiration provide similar residence times for TOC as we find for the neutral sugar fraction of HMW DOC and suggest that our radiocarbon results may apply to a large fraction of the semireactive HMW DOC.

Neutral sugars represent 15–20% of the total HMW DOC, and at least 5–7% of total DOC. Although HMW DOC is more enriched in radiocarbon than total DOC, and therefore enriched in the semireactive component, our results apply to only a portion of the total semireactive DOC fraction. Further measurements are needed to determine if other fractions of semireactive DOC have the same radiocarbon values as neutral sugars, and the extent to which semireactive DOC fuels bacterial production. If a large fraction of semireactive DOC cycles on timescales of <1–3 yr, semireactive DOC meets an important fraction of global marine bacterial carbon demand.

Compound-specific radiocarbon analyses and DOC cycling in the mesopelagic ocean—Neutral sugar concentrations decrease from 4–6 μmol L^{-1} C or 13–21% of HMW DOC in surface samples to 0.7μmol L^{-1} C or 6% of HMW DOC at 600 m. Despite the order-of-magnitude decrease in neutral sugar concentration, NMR spectra and molecular-level sugar analyses show the APS portion of HMW DOC to be remarkably homogeneous in composition between all three samples (Fig. 4). HMW DOC samples collected in the NPSG all show the presence of carbohydrate, acetate, and alkyl carbon in the ^{1}H NMR spectra, as well as the suite of seven neutral sugars characteristic of APS even to depths of 5,200 m (Fig. 4). The carbohydrate fraction of HMW DOC can be introduced into the mesopelagic ocean through two fundamentally different mechanisms. A small fraction of the reactive carbohydrate synthesized in the euphotic zone may escape degradation and be mixed into the mesopelagic ocean by advection (Hansell et al. 2002). These relic sugars will age at the same rate as DIC, and have a radiocarbon value equal to DIC at depth. Alternatively, sugars could be introduced from the dissolution of rapidly sinking large particles (Engel et al. 2004). Reactive DOC injected by sinking particles will have radiocarbon values similar to surface-water DIC.

To distinguish these two mechanisms, we compared radiocarbon values of DIC and neutral sugars in samples from 600 m. The $\Delta^{14}C$ of DIC and HMW DOC at 600 m sample are −155 ± 7‰ and −258‰ respectively, and are typical of values at this depth in the North Pacific Ocean. Neutral sugars at 600 m have radiocarbon values between −108 and −133‰, and are enriched by up to 150‰ relative to HMW DOC. The average $\Delta^{14}C$ value obtained by treating glucose, galactose, xylose, and mannose as replicates is −123 ± 10‰ (1 SD, n = 4), and is slightly enriched in radiocarbon relative to DIC (−155 ± 7‰). Our data suggest that some

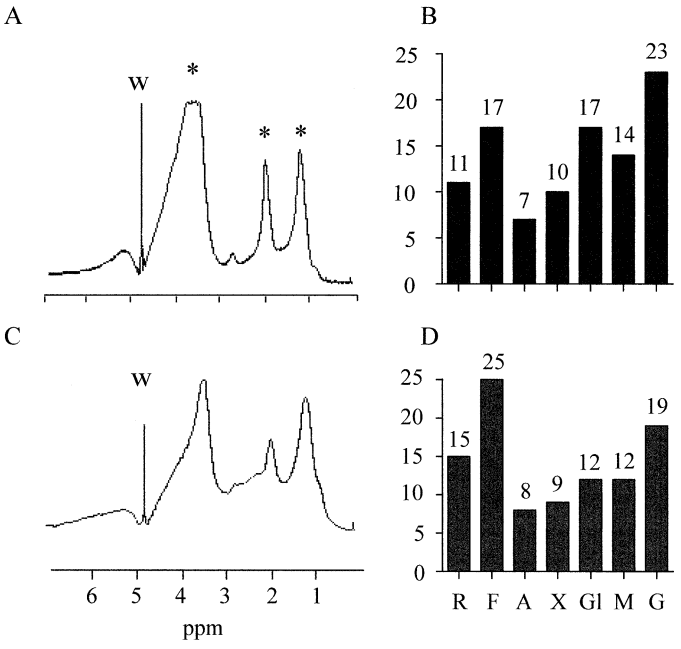

Fig. 4. (A, B) ^{1}H NMR spectra and monosaccharide distribution of HMW DOC from surface and (C, D) 5,200 m seawater collected at the NPSG site. The ^{1}H NMR spectra of HMW DOC at both depths display prominent peaks (*) for carbohydrate (3–4.5 ppm), acetate (2 ppm), and alkyl carbon (1.3 ppm). The sharp peak at 4.8 ppm is water, which was used as a solvent and reference. The monosaccharide distribution was determined by gas chromatography of alditol acetates according to methods described in Aluwihare et al. (2002). Acid hydrolysis of each sample releases seven major neutral sugars: rhamnose (R), fucose (F), arabinose (A), xylose (X), glucose (Gl), mannose (M), and galactose (G). Numbers above each bar in the figure correspond to the percentage of that sugar relative to the total seven neutral sugars. The 5,200 m sample is somewhat enriched in fucose relative to the surface sample, but otherwise contains the same suite of sugars in approximately the same relative amounts as surface seawater HMW DOC.

fraction of neutral sugars might be introduced by the dissolution of rapidly sinking particles. If we assume that neutral sugars at 600 m are a simple mixture of newly synthesized sugars (introduced by sinking particles) with a $\Delta^{14}C$ value equal to surface water DIC, and relic sugars (introduced by advection) with a $\Delta^{14}C$ value equal to DIC $\Delta^{14}C$ at 600 m depth, then 15% of the neutral sugars at 600 m are introduced by large, rapidly sinking particles. These sugars may be reactive and help support bacterial activity at depth (Arístegui et al. 2002). A more comprehensive set of radiocarbon measurements on neutral sugars from depth in the ocean is needed to fully establish the isotopic differences between DIC and reactive components of HMW DOC.

Compound-specific radiocarbon analysis offers a geochemical tracer approach to explore carbon cycling within the DOC reservoir. Using this approach we find that the neutral sugar fraction of HMW DOC has a residence time in the euphotic zone of <25 yr and perhaps as short as <3 yr. The similarity in NMR spectra, neutral sugar yields, and distributions in HMW DOC in the upper ocean suggest that our measurements of neutral sugar $\Delta^{14}C$ values may be representative of a much larger fraction of HMW DOC. Our data also support other measurements that suggest that semireactive DOC may play an important role in meeting bacterial carbon demand but radiocarbon analyses of other compound classes (amino acids, amino sugars) are needed to provide a more comprehensive inventory of reactive DOC components and residence times. Finally, the data presented in this paper suggest that compound-specific radiocarbon analyses may provide an avenue to distinguish delivery processes for reactive fractions of DOC in the meso- and bathypelagic ocean.

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